Variation in the Vertebral Number of the Anchovy (*Stolephorus purpureus*) in Hawaiian Waters[1]

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A STUDY OF VARIATION in vertebral number of the nehu, *Stolephorus purpureus* Fowler, from various localities throughout the Territory of Hawaii, has been undertaken in an effort to determine whether one or several populations of this small but valuable baitfish exist. Large quantities of nehu are caught each year on several different baiting grounds and are used in livebait fishing for the aku or skipjack. A recent scarcity of bait in some areas has posed the question of whether the stock is being overfished. Therefore, knowledge of whether each baiting area supports a separate population is of practical importance as well as of academic interest.

METHODS

Sampling

The study is based on 18 samples taken mostly from the commercial catch. Although efforts were made to secure random samples, some of those submitted by the fishermen may include a disproportionate number of smaller specimens, if, as is likely, they furnished fish which died in their baitwells. However, this would not influence the conclusions which have been drawn.

Some of the samples were taken from catches made at night by night-light nets; others were taken from catches made in the daytime by surround nets. These methods of fishing have been described by June (1951).

Seven different localities are represented: Ala Wai Canal, Honolulu Harbor, Pearl Harbor, and Kaneohe Bay on the island of Oahu; Kihei on the island of Maui; and Hilo Harbor and Kawaihae Bay on the island of Hawaii (Fig. 1). Unfortunately no samples were obtained from other important baiting grounds on the islands of Kauai and Molokai.

The samples were collected as occasion permitted over a period of years, from 1944 to 1949. There was no particular pattern as to time of collection or number examined. All samples were preserved in approximately 10 per cent formaldehyde.

Preparation of Vertebral Columns

Two methods of preparing the vertebral columns for counting were used, the "photographic" and the "direct." In the photographic method the fish were subjected to the following procedure (a modified Spalteholz-type preparation):

(1) Wash in running water for 3 hours; or let stand in still water for 5 hours, changing the water every hour.

(2) Remove viscera, scales, and, in larger specimens, gills.

(3) Bleach for 1 to 3 hours in 3 per cent hydrogen peroxide until lateral line and peritoneum are clear.

(4) Wash for 1 hour in running water.

(5) Place in 1 per cent potassium hydroxide for a half hour and aspirate with water-vacuum pump to remove air bubbles from fish.

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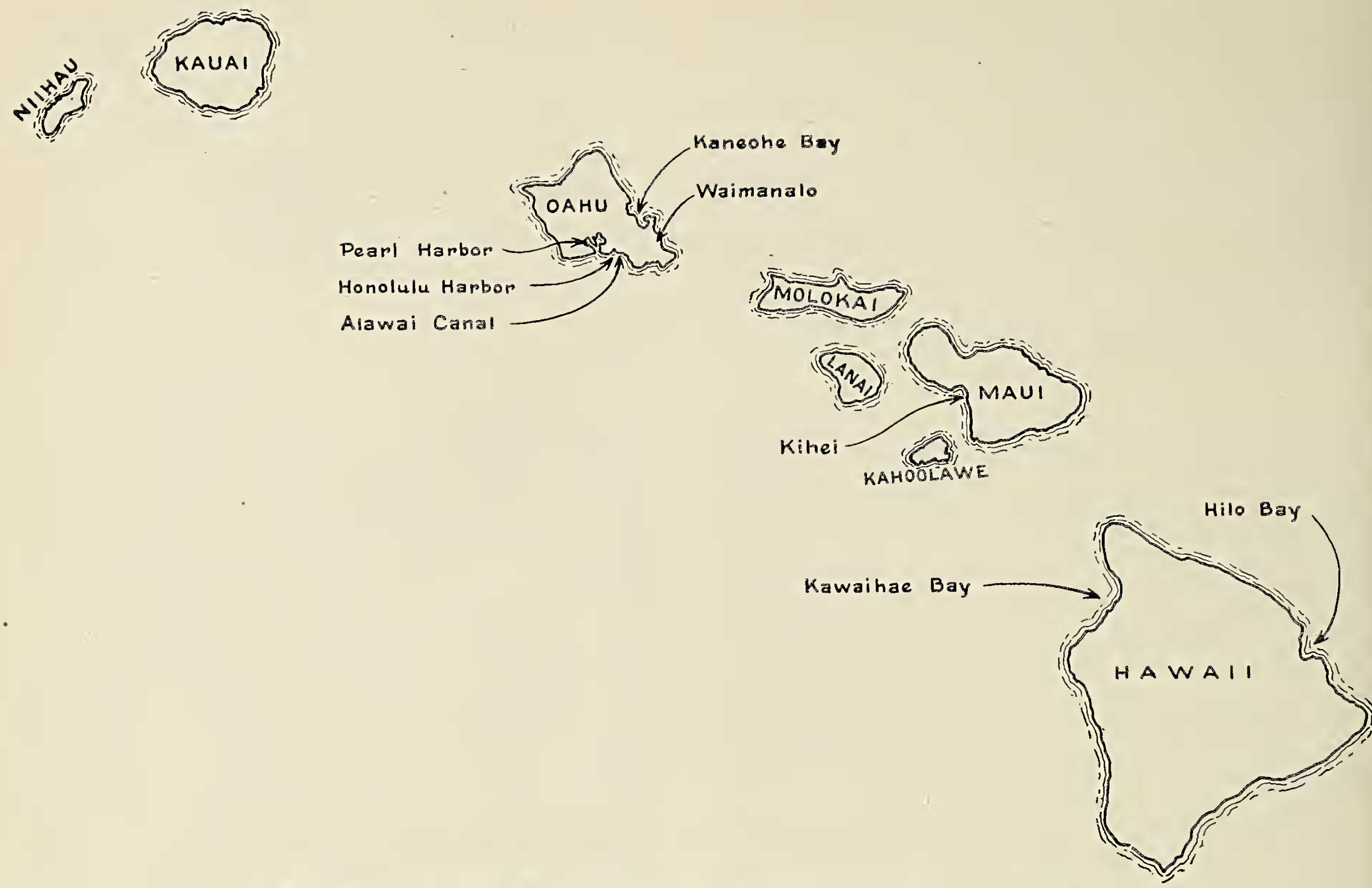

FIG. 1. Map of the Hawaiian Islands showing the localities sampled.

(6) Place in 4 per cent potassium hydroxide for 4 to 6 hours, until vertebrae can be seen clearly.

(7) Place in alizarin staining solution (see below), stain for 10 hours, then turn the fish over and stain the other side for 5 to 10 hours.

(8) Place, successively, in a series of 4 per cent potassium hydroxide–glycerin solutions (90:10; 80:20; 60:40; 20:80; 0:100), each for 12 hours; if a solution turns dark red-blue, repeat that particular solution.

(9) Preserve in 100 per cent glycerin, adding two or three small crystals of thymol to prevent the growth of molds; all parts of the fish, except the bones, are now transparent.

(10) Place the fish in a container filled with glycerin and, using a standard Kodak enlarger, project the image on photographic paper, expose, and develop (Fig. 2); several fish may be photographed simultaneously.

(11) Using the photograph, count the number of vertebrae.

In the direct method, the fish were prepared in the following manner:

(1) Wash in running water for 1 to 3 hours.

(2) Using a scalpel, slice the flesh from the caudal peduncle to the head on the left side of the fish, cutting to, but not into, the column; using scissors, snip the flap of flesh diagonally across the head, thus exposing the base of the skull and the first vertebra; using scalpel, scrape the remaining flesh from the column on the left side of the fish and remove viscera from body cavity.

(3) Place the fish in staining solution for 4 hours.

(4) Transfer to water or to 5 per cent formaldehyde solution; some de-staining will occur.

(5) Count the vertebrae under the low-power binocular microscope, using a pointer (eyelash hair) in the ocular as a reference point.

A stock solution of stain was made by dis-

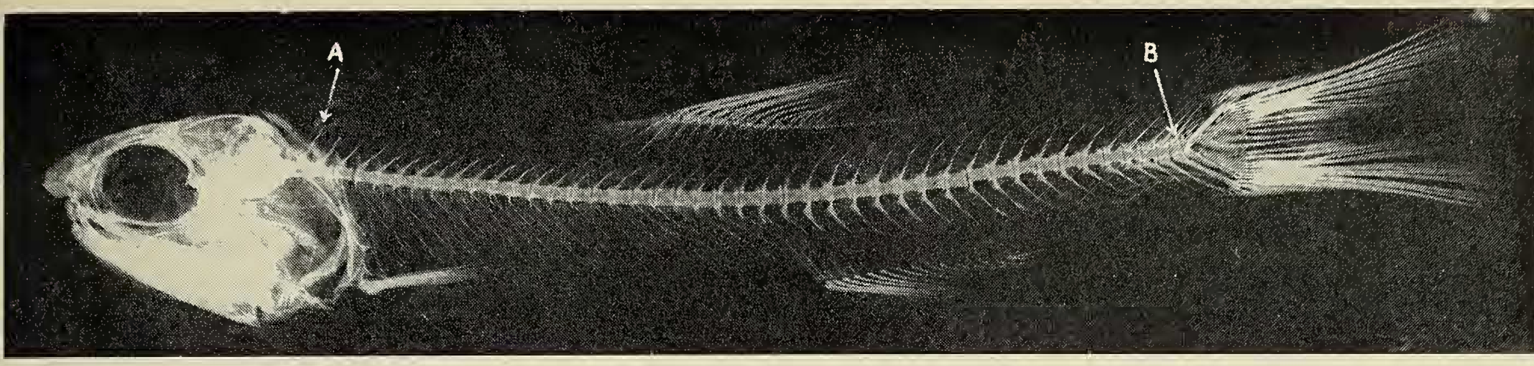

Fig. 2. Vertebral column prepared by the "photographic" method. Note the anterior (A) and posterior (B) end points in counting.

solving 1 gram of Alizarine Red S. in 500 milliliters of 3 per cent potassium hydroxide solution. The staining solution consisted of 10 milliliters of the stock solution added to 100 milliliters of 3 per cent potassium hydroxide.

In counting the vertebrae, both end points were clearly defined. The first vertebra was readily identified by its high spine with a small triangle of cartilage *both* anterior and posterior to it. The last vertebra was identified as the one immediately anterior to the upturned, segmented urostyle. These end points may be seen in Figure 2. Thus the count, which was checked at least once for each fish, included neither the basioccipital nor the urostyle.

Both methods of preparation were satisfactory. However, the first method was time consuming, and in several cases it was difficult to get the column in clear focus along its entire length for photographing. The second method is recommended. With its use, a small number of vertebral columns with abnormal centra (6 cases out of 851) were detected, and the counts on these abnormal specimens were discarded.

Length Measurements

Length measurements were made on all samples, but in one from Kaneohe Bay (10/25/47), they were not identified with vertebral number. Standard length is defined as the distance (measured to the nearest millimeter) from the tip of the upper jaw to the posterior edge of the hypural plates. In specimens which were photographed, the length was determined with dividers and was measured on a scale which was photographed with the fish. For specimens in which the vertebrae were counted under the microscope, the length was measured directly with vernier callipers.

RESULTS

In a sample from Kaneohe Bay (10/25/47) in which the sexes were segregated, the mean vertebral number for 76 males was 42.618 and for 76 females, 42.645. The difference of 0.027 had a standard error of 0.095. A difference of this magnitude could arise readily from chance variation. It was concluded that sex need not be considered as a source of variation in mean vertebral number. This conclusion is in accord with those found by most workers in other species, e.g., Tester (1937) in Pacific herring (*Clupea pallasii*) and Blackburn (1950) in the Australian anchovy (*Engraulis australis*), but it is in contrast with that found by Hart (1937) for the Pacific capelin (*Mallotus villosus*).

In the Pacific herring (McHugh, 1942), there is a tendency for the mean vertebral number to be higher in larger fish, a phenomenon which is presumed to be related to hereditary differences in the developmental rate of individuals. To investigate this possibility in the nehu, the fish of each sample were grouped in 3-millimeter-length categories and the mean count for each was determined. The results, for length categories including 10 or more fish, are shown in Table 1 and Figure

3A. In the figure, the mean count of each length group is plotted as a deviation from the mean count of the sample.

It will be noted from Figure 3A that, in some samples, mean vertebral number tends to increase with length, in others it tends to decrease, and in still others it shows no apparent relationship. Using the original ungrouped data it was found that either positive or negative regressions of vertebral number on length could be statistically significant. For example, in a sample from Ala Wai Canal (5/22/44) the positive regression coefficient of 0.0222 over all lengths had a standard error of 0.00863 and differed significantly from zero (P = 0.02). Also, in a sample from Honolulu Harbor (3/3/49) the negative regression coefficient of —0.0355 over length groups 24 to 40 millimeters (Fig. 3A) had a standard error of 0.0162 and differed significantly from zero (P = 0.04).

Before attempting to interpret the above results, it should be pointed out that the spawning period of the nehu in those localities which have been investigated (Ala Wai Canal and Kaneohe Bay) extends throughout the entire year. Moreover, the period between fertilization and hatching of the egg is very short—24 hours or less.

If, as in many other plastic species, vertebral number is influenced by environmental conditions obtaining during the time of embryonic and early larval development, variation in environmental conditions from day to day might produce variation in the mean vertebral number of successive day-broods. In some species, e.g., herring—*Clupea pallasii* (Tester, 1938)—there seems to be an inverse relationship between mean vertebral number and water temperature during the period of early development, low mean counts being associated with high temperatures, and vice versa. In others, e.g., the Pacific anchovy—*Engraulis mordax* (Hubbs, 1925)—there seems to be a direct relationship between mean vertebral number and salinity, the low mean counts being associated with less saline, brackish water conditions, and vice versa. Therefore, in the nehu, upward or downward trends in temperature or salinity, extending over a period of days or weeks, might produce downward or upward trends in mean vertebral number within the length range of a sample, thus explaining the occurrence of both positive and negative regressions. It is not necessary to postulate an inherited tendency for fast-growing, larger individuals of one brood to have a higher count than slow-growing, smaller individuals of the same brood, although the tendency may still exist.

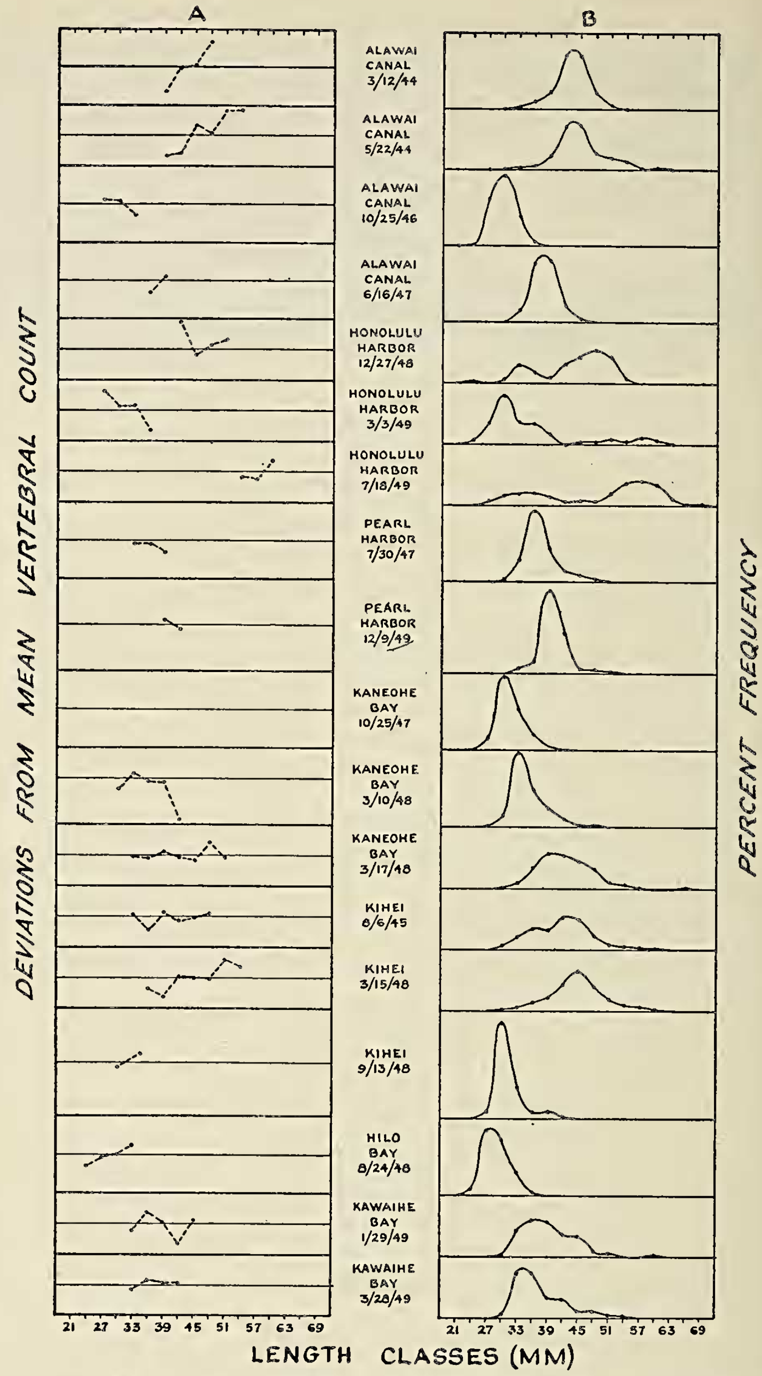

FIG. 3. A, Variation in mean vertebral count with length; B, length frequency distributions of the samples.

The length frequency distributions of the

TABLE 1

VERTEBRAL COUNTS OF NEHU, WITH MEANS ACCORDING TO LENGTH GROUPS (10 OR MORE SPECIMENS, ONLY) AND SAMPLES (ALL SPECIMENS)

LOCALITY AND DATE	LENGTH GROUP	NUMBER OF FISH WITH COUNTS OF 40	41	42	43	44	TOTAL NUMBER	MEAN FOR LENGTH GROUP	MEAN FOR ENTIRE SAMPLE
Ala Wai Canal, Oahu 3/12/44	39	1	7	3	–	–	11	41.182	
	42	2	14	19	–	–	35	41.486	
	45	2	14	18	1	–	35	41.514	
	48	–	3	7	1	–	11	41.818	
	All	5	43	50	2	–	100	—	41.490
Ala Wai Canal, Oahu 5/22/44	39	2	14	3	2	–	21	41.238	
	42	3	40	19	1	–	63	41.286	
	45	2	21	40	1	–	64	41.625	
	48	2	9	13	1	–	25	41.520	
	51	–	4	12	1	–	17	41.824	
	54	1	3	6	3	–	13	41.846	
	All	13	106	110	12	–	241	—	41.502
Ala Wai Canal, Oahu 10/25/46	27	–	12	20	3	–	35	41.743	
	30	1	15	34	2	–	52	41.712	
	33	1	9	11	1	–	22	41.545	
	All	3	37	66	7	–	113	—	41.681
Ala Wai Canal, Oahu 6/16/47	36	1	8	10	–	–	19	41.474	
	39	–	10	9	1	–	20	41.550	
	All	2	22	24	1	–	49	—	41.490
Honolulu Harbor, Oahu 12/27/48	33	–	1	7	2	–	10	42.100	
	42	–	–	3	8	–	11	42.727	
	45	–	1	9	5	–	15	42.267	
	48	–	–	12	6	1	19	42.421	
	51	–	–	8	7	–	15	42.467	
	All	–	2	54	31	–	88	—	42.352
Honolulu Harbor, Oahu 3/3/49	27	–	–	1	11	2	14	43.071	
	30	–	1	6	25	3	35	42.857	
	33	–	–	4	10	2	16	42.875	
	36	–	–	7	8	–	15	42.533	
	All	–	1	26	74	7	108	—	42.806
Honolulu Harbor, Oahu 7/18/49	54	–	1	8	7	–	16	42.375	
	57	–	1	9	7	–	17	42.353	
	60	–	–	6	9	–	15	42.600	
	All	–	8	46	50	3	107	—	42.449
Pearl Harbor, Oahu 7/30/47	33	–	–	10	2	–	12	42.167	
	36	–	3	26	9	–	38	42.158	
	39	–	4	9	5	–	18	42.055	
	All	–	7	52	24	–	83	—	42.205

TABLE 1 (*cont'd*)

LOCALITY AND DATE	LENGTH GROUP	NUMBER OF FISH WITH COUNTS OF 40	41	42	43	44	TOTAL NUMBER	MEAN FOR LENGTH GROUP	MEAN FOR ENTIRE SAMPLE
Pearl Harbor, Oahu 12/9/49	39	–	1	30	28	3	62	42.532	
	42	–	1	16	13	–	30	42.400	
	All	–	2	57	50	4	113	—	42.496
Kaneohe Bay, Oahu 10/25/47	All	–	12	262	335	16	625	—	42.568
Kaneohe Bay, Oahu 3/10/48	30	–	–	7	11	–	18	42.611	
	33	–	2	27	80	7	116	42.793	
	36	–	1	18	38	3	60	42.717	
	39	–	–	10	19	1	30	42.700	
	42	–	–	5	6	2	13	42.231	
	All	–	3	68	159	14	244	—	42.754
Kaneohe Bay, Oahu 3/17/48	33	–	–	3	7	–	10	42.700	
	36	–	–	13	18	2	33	42.667	
	39	–	–	18	34	4	56	42.750	
	42	–	3	13	35	2	53	42.679	
	45	–	–	17	25	1	43	42.628	
	48	–	–	7	21	3	31	42.871	
	51	–	–	3	7	–	10	42.700	
	All	–	3	79	151	13	246	—	42.707
Kihei, Maui 8/6/45	33	–	–	3	7	–	10	42.700	
	36	–	–	9	6	1	16	42.500	
	39	–	–	5	9	1	15	42.733	
	42	–	–	9	15	–	24	42.625	
	45	–	–	8	13	1	22	42.682	
	48	–	–	4	6	1	11	42.727	
	All	–	–	45	65	6	112	—	42.688
Kihei, Maui 3/15/48	36	–	–	6	7	–	13	42.538	
	39	–	–	13	10	–	23	42.435	
	42	–	–	17	24	3	44	42.682	
	45	–	–	23	39	2	64	42.672	
	48	–	–	15	22	2	39	42.667	
	51	–	–	4	13	2	19	42.895	
	54	–	–	2	9	–	11	42.818	
	All	–	–	85	134	9	228	—	42.667
Kihei, Maui 9/13/48	30	–	–	24	36	6	66	42.727	
	33	–	–	6	11	4	21	42.905	
	All	–	–	33	59	12	104	—	42.798
Hilo Bay, Hawaii 8/24/48	24	–	1	6	5	1	13	42.462	
	27	–	–	54	64	3	121	42.579	
	30	–	3	41	55	6	105	42.610	
	33	–	–	13	29	1	43	42.721	
	All	–	4	117	155	12	288	—	42.608

TABLE 1 (*cont'd*)

LOCALITY AND DATE	LENGTH GROUP	NUMBER OF FISH WITH COUNTS OF 40	41	42	43	44	TOTAL NUMBER	MEAN FOR LENGTH GROUP	MEAN FOR ENTIRE SAMPLE
Kawaihae Bay, Hawaii 1/29/49	33	–	–	12	12	1	25	42.560	
	36	–	–	9	24	2	35	42.800	
	39	–	–	13	18	2	33	42.667	
	42	–	–	14	6	1	21	42.381	
	45	–	–	8	10	2	20	42.700	
	All	–	–	59	77	9	145	—	42.655
Kawaihae Bay, Hawaii 3/28/49	33	–	–	12	20	4	36	42.778	
	36	–	1	4	28	3	36	42.917	
	39	–	–	4	10	2	16	42.875	
	42	–	1	3	8	3	15	42.867	
	All	–	2	29	78	14	123	—	42.845

samples are shown in Figure 3B, the number in each length category being represented as a percentage of the total number in the sample. There is considerable variation between samples in both the length range and the position of the mode. Two of the samples, both from Honolulu Harbor, have at least two modes. In the first (12/27/48) the mean vertebral number for the smaller modal group (less than 39 mm.) is 42.136, and for the larger modal group (greater than 39 mm.), 42.431. The difference of 0.295 has a standard error of 0.133 and is significant (P = 0.03). In the second (7/18/49) the mean of the smaller group (less than 45 mm.) is 42.459 and of the larger group (greater than 45 mm.), 42.443. In this case, the difference of 0.016 has a standard error of 0.146 and is not significant. Again, differences such as these, if real, might be induced by differences in environmental conditions during the period of early development.

In the preceding paragraphs it has been shown that variation other than that due to chance may occur between length groups within samples. Accordingly, more generally to assess its significance and to allow for it in comparisons between samples, this source of variation was included in an analysis of variance of the data, considering the sampling to have been completely randomized. The analysis, limited to length groups containing 10 or more individuals, is summarized in the following tabulation:

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F
Localities	6	451.2679	75.2113	37.59**
Samples	10	20.0099	2.0010	4.09**
Length groups	55	26.8984	0.4891	1.39*
Individuals	2119	743.3730	0.3508	

** Highly significant; P is 0.01 or less.
* Significant; P is 0.05 or less, but greater than 0.01.

From a comparison of these F values with those tabulated for homogeneous distributions by Snedecor (1946: 10.4), it will be found that significant heterogeneity occurs between length groups of the same sample, between samples of the same locality, and between localities. The component of variance associated with "length groups" is obviously small. The conclusion for "samples" would have remained the same if the sum of squares and degrees of freedom for "length groups" and "individuals" had been pooled, and the resultant mean square used as the error term in testing this next higher category. In view of this, a second analysis, which does not allow for variation between length groups but which includes all the data rather than only some of them, was undertaken with the following results:

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F
Localities	6	520.2088	86.7015	31.97**
Samples	11	29.8275	2.7116	7.61**
Individuals	3099	1103.7526	0.3562	

** Highly significant; P is 0.01 or less.

As in the previous analysis, it is apparent that there are significant differences between the means for samples from the same locality and between the means for localities. The various means are tabulated in Table 2.

Heterogeneity between the means of samples from the same locality arises chiefly from the material taken at Honolulu Harbor and Kaneohe Bay. In the former, the mean of one sample (42.806) differs significantly from those of the other two (42.352 and 42.449). In the latter, the mean of one sample (42.568) also differs significantly from those of the other two (42.754 and 42.707). Differences such as these may represent: (1) the presence of two or more genetically separate stocks within a locality; (2) the mixture of stocks between localities; or (3) seasonal variation in environmental conditions within a locality, the stock remaining discrete.

Regarding (1), although the possibility of genetically separate stocks occurring within a locality cannot be ruled out, it seems unlikely that these would occur in fish, such as the nehu, which have pelagic eggs, and which spawn in partially enclosed waters with good circulation and mixing. Regarding (2), the possibility of schools migrating from one baiting area to another cannot be denied. However, as will be brought out in the discussion, there is little evidence that nehu schools occur commonly in offshore waters. Regarding (3), there is a difference of about 3°C. between summer and winter water temperatures within a locality. This, or seasonal differences in salinity, or a combination of both, may be sufficient to induce the differences in mean count between samples within localities.

The analysis-of-variance tables show that there is highly significant heterogeneity between localities, allowing for the variation between samples from the same locality. Inspection of the data (Table 2) indicates that the heterogeneity stems mostly from material for the island of Oahu. The mean for Ala Wai Canal (41.539) is outstandingly low as compared with the means for all other localities. To determine whether it differs significantly from those of other Oahu localities, the mean square for "samples" was accepted as a common variance (Snedecor, 1948: 10.4), thus leading to a calculation of the fiducial interval ($P = 0.05$) of a locality mean, which amounts to ± 0.279. The upper "limit" for Ala Wai Canal (41.818) is much less than the lower "limit" for Pearl Harbor (42.093), the locality with the closest mean count. It seems certain that the Ala Wai Canal fish constitute a distinct statistical group in respect to vertebral number, and that they mix little, if at all, with the fish of other localities.

The striking difference between the mean vertebral number of Ala Wai Canal nehu and those of other localities may be due to genetic factors, environmental factors, or both. The probability that the difference is of genetic origin could be investigated only by conducting rearing experiments on Ala Wai Canal fish and those of other areas under controlled conditions. The possibility that the difference is of environmental origin may be investigated with such data as are on hand. Surface temperatures and chlorinities over the period February to June, 1949, are available for three stations in both Ala Wai Canal and Kaneohe Bay. Average determinations, made within 1 or 2 days of each other, are shown in Table 3. During this period, Ala Wai Canal had a temperature which averaged about 0.7°C. higher and a chlorinity which averaged about 2.4 ppm lower than Kaneohe Bay. These, or even greater differences, are to be

TABLE 2

Summary of Vertebral Data. Numbers of Fish and Mean Counts According to Sample, Locality, and Island

LOCALITY AND DATE	SAMPLE		LOCALITY		ISLAND	
	number	*mean*	*number*	*mean*	*number*	*mean*
Ala Wai Canal, Oahu						
3/12/44	100	41.490	503	41.539	2117	42.340
5/22/44	241	41.502				
10/25/46	113	41.681				
6/16/47	49	41.490				
Honolulu Harbor, Oahu						
12/27/48	88	42.352	303	42.548		
3/3/49	108	42.806				
7/18/49	107	42.449				
Pearl Harbor, Oahu						
7/30/47	83	42.205	196	42.372		
12/9/49	113	42.496				
Kaneohe Bay, Oahu						
10/25/47	625	42.568	1115	42.639		
3/10/48	244	42.754				
3/17/48	246	42.707				
Kihei, Maui						
8/6/45	112	42.688	444	42.703	444	42.703
3/15/48	228	42.667				
9/13/48	104	42.798				
Hilo Bay, Hawaii						
8/24/48	288	42.608	288	42.608	556	42.673
Kawaihae Bay, Hawaii						
1/29/49	145	42.655	268	42.743		
3/28/49	123	42.845				

TABLE 3

Mean Temperature (°C.) and Chlorinity (ppm) at Three Stations in Ala Wai Canal and Kaneohe Bay for Corresponding Times

ALA WAI CANAL			KANEOHE BAY			DIFFERENCE	
Date	Temperature	Chlorinity	Date	Temperature	Chlorinity	Temperature	Chlorinity
2/4/49	24.6	13.62	2/3/49	23.3	17.42	1.3	−3.80
2/15/49	23.5	15.61	2/17/49	23.1	17.14	0.4	−1.53
3/7/49	24.7	16.56	3/8/49	23.5	17.19	1.2	−0.63
3/16/49	24.3	17.47	3/15/49	22.6	18.07	1.7	−0.60
3/21/49	24.3	17.19	3/22/49	23.8	17.57	0.5	−0.38
3/30/49	24.3	16.26	3/29/49	24.0	17.72	0.3	−1.46
4/13/49	24.2	13.15	4/12/49	23.1	18.24	1.1	−5.09
4/27/49	24.2	16.86	4/26/49	23.8	18.39	0.4	−1.53
5/2/49	24.7	17.47	5/3/49	24.8	18.73	−0.1	−1.26
5/16/49	26.7	16.82	5/17/49	26.0	19.47	0.7	−2.65
5/23/49	26.2	11.75	5/24/49	25.8	19.15	0.4	−7.40
6/8/49	26.3	16.20	6/7/49	26.0	18.22	0.3	−2.02
Mean difference						0.7	−2.38

expected, as Ala Wai Canal is a narrow inlet with a small opening to the sea and with a relatively large stream entering near its head, whereas Kaneohe Bay is a large body of water with a wide exposure to the sea and with a relatively small volume of fresh water entering it from a few streams. As the average difference in temperature between localities (0.7°C.) is much less than the average difference between summer and winter temperatures within localities (about 3° C.), it would appear that temperature alone could not account for the large difference in mean count between Ala Wai Canal (41.539) and Kaneohe Bay (42.639), although it is in the direction which might be expected.

Differences in mean vertebral count which are apparently related to differences in salinity have been reported by Hubbs (1925) for the Pacific Coast anchovy (*Engraulis mordax*), as noted above, and also by Blackburn (1950) for the Australian anchovy (*Engraulis australis*). In the former, the fish in the brackish waters of San Francisco Bay had a much lower mean count (43.80) than those near the entrance of the bay and seaward from the entrance (45.73). Similarly, in the latter, the fish in the brackish waters of the rivers of Gippsland lakes also had a lower mean count (44.56) than those near the entrance to the sea (45.20). Thus, the strikingly different mean count for the Ala Wai Canal fish is probably related in large part to the brackish-water habitat.

Inspection of Table 2 shows that not only is the mean for Ala Wai Canal (41.539) outstandingly low, but also that the means for other localities on the leeward shore of Oahu —Pearl Harbor (42.372) and Honolulu Harbor (42.548)—are lower than that of Kaneohe Bay (42.639) on the windward shore. One might be tempted to the conclusion that there are also distinct populations in these other localities. Consideration of their relative sizes, extent of exposure to the open sea, fresh-water supply, etc., would suggest that Oahu localities range in the following order of increasing average salinity: Ala Wai Canal, Pearl Harbor, Honolulu Harbor, Kaneohe Bay. This order conforms with progressively increasing mean count: 41.539, 42.372, 42.548, 42.639. However, the fiducial intervals of the means for all localities other than Ala Wai Canal overlap considerably. Therefore, they could have arisen in random sampling from one complex population.

The mean vertebral counts for localities on the other islands also could, with some speculation, be fitted into the above gradation. Certainly, in the absence of more precise information, Kawaihae Bay, with the highest mean count (42.743), would be placed at the opposite end of the series to Ala Wai Canal, as it is open to the sea and has only one or two very small streams flowing into it which are dry during part of the year. Again, however, there is no statistical evidence for the segregation of the mean counts of these other localities.

The lack of significant differences between the means for localities other than Ala Wai Canal is more clearly demonstrated in the analysis of variance which follows. In this, the Ala Wai Canal data have been omitted. It will be observed that there is no longer significant heterogeneity between localities:

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F
Islands	2	5.9321	2.9661	
Localities	3	15.0686	5.0229	1.50
Samples	8	26.8484	3.3560	9.78**
Individuals	2600	891.7377	0.3430	

** Highly significant; P is 0.01 or less.

In the foregoing analysis, an additional category—islands—has been incorporated. It will be observed that there are no significant differences between the means for islands (Table 2). In fact, the mean square for islands is considerably less than might have been anticipated. This may be due to the small number of localities which were sampled on islands other than Oahu. No conclusions as to whether or not the nehu of the various islands mix can be drawn from the analysis of the vertebral material.

DISCUSSION

The nehu is characteristically an inshore fish. For the most part, it is believed to occur only in certain isolated localities where the water is relatively more turbid and less saline than that of the open sea. As yet, it has not been seen at the outer edges of the reefs during numerous skin-diving expeditions, nor has it been taken by poisoning in these localities. However, the fact that the fish may be kept alive in the baitwells of vessels for several days in offshore waters shows that they can live, for a time at least, under ocean conditions. Moreover, there is one report of nehu having been seen in quantity outside the reef area. Mr. Lester Zukeran, a former fisherman and presently skipper of the University's research vessel *Salpa*, reports that, in the summer of 1940, large schools of nehu were present about a mile offshore, from Kaneohe Bay to Waimanalo on the windward shore of Oahu. They were so abundant that they interfered with fishing, the tuna failing to respond to the bait when it was thrown overboard. As the nehu were of large size, larger than those caught in Kaneohe Bay, the fishermen advanced the theory that they had migrated from Kihei, Maui, where large fish were commonly encountered. This is believed to be an unusual occurrence of nehu in outside waters. If it were common, it is reasonably certain that other reports would have come to our attention, for there are many tuna fishermen operating in outside waters during all months of the year. On the basis of this information, the hypothesis might be advanced that an essential, discrete population of nehu occurs in each separate baiting area, but with the provision that, on occasion, some mixing may occur between the stocks of the different areas.

The present analysis of vertebral data gives some support to this hypothesis, but not as much as might be desired for its adoption as a basis for regulation of the fishery. The existence of a separate population has been shown for one area only, Ala Wai Canal. For the others, the difference in mean vertebral count between localities could have arisen in random sampling from one statistically complex biological population. There is some indication of the presence of essentially separate units from the correspondence between the order of increasing mean counts for localities and the order of increasingly saline conditions within the localities, but the latter were estimated and not measured.

Although the hypothesis that essentially discrete units occur in each baiting area has not received a great deal of support from the analysis of vertebral material, it has not been disproved. More extensive sampling might demonstrate that there are small, but real, differences in mean count between localities and between islands, apart from the known heterogeneity between samples from one locality. Further evidence regarding the intermixture or isolation of populations, other than that of Ala Wai Canal, must await future investigations, preferably by some other method of study.

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